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Production of Monoclonal Antibodies to Flagellar Core Protein of *Vibrio parahaemolyticus*

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ABSTRACT

Polar flagellar core protein was purified from *Vibrio parahaemolyticus* by differential centrifugation and cesium chloride isopycnic centrifugation. Twelve hybridomas secreting monoclonal antibodies against flagellar core protein (MAb-flc) were obtained from fusions. By chance, one purified flagellar core preparation retained flagellar sheath and as a consequence, two hybridomas were detected which secreted anti-sheath antibodies (MAb-fls). MAb-flc and MAb-fls reacted specifically with their corresponding antigens as demonstrated by immunogold labelling. Coagglutination reagents prepared with MAb-flc and MAb-fls were tested against 35 strains of *V. parahaemolyticus* and 34 heterologous *Vibrio* species. The coagglutination results revealed that the flagellar core was species-specific while the flagellar sheath was not.

Key words : *Vibrio parahaemolyticus*, flagellar core protein, flagellar sheath, monoclonal antibody.

INTRODUCTION

V. parahaemolyticus is a Gram-negative, rod-shaped, straight or slightly curved bacterium (Bauman *et al.*, 1984). Strains described in the United States and Japan share the following characteristics: they are Gram-negative, facultatively anaerobic rods exhibiting pleomorphism and motility by polar monotrichous and peritrichous or lateral flagella (Rieman and Bryan, 1979). The single polar flagellum is produced when the bacterium is propagated in liquid or on solid medium (Bauman *et al.*, 1971). The lateral flagella were produced only under the following culture conditions (Allen and Bauman, 1971; Bauman *et al.*, 1971; Bauman *et al.*, 1973); when grown on agar plates, embedded in solidified medium, suspended in viscous medium or agglutinated with antibody in liquid media. The lateral flagella can be easily removed mechanically, whereas the polar flagellum can be removed only following rigorous agitation of the cells (Miwatani *et al.*, 1970). The polar flagellum is encased in a sheath-like structure, whereas lateral flagella are not (Allen and Bauman, 1971; Miwatani *et al.*, 1970).

Isolated flagellin monomers of the polar flagella were analysed on hydroxylapatite column and two distinct peaks were detected. One was eluted with 0.03 M phosphate buffer and the other with 0.1 M phosphate buffer. These subunits were tentatively designated as U-I and U-II, respectively.

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On a Sephadex G-100 column, each flagellin subunit showed a similar molecular weight of 40 kilodalton (kD). Examination of the physical and chemical properties of the subunits revealed that the U-I and U-II differed in their amino acid composition (Shinoda *et al.*, 1970).

Antisera raised in rabbits immunized with flagellin proteins of *V. parahaemolyticus* polar flagellum (PH) were tested by gel diffusion assay. Serological similarity of the anti-flagellin protein to the crude flagellin prepared from several *Vibrio* species was obtained as following: *V. alginolyticus*, *V. anguillarum*, *V. campbellii*, *V. cholerae*, *V. ichthyodermis*, *V. natreigens*, *V. neptuna*, *V. nereida*, *V. pelagia* and *V. piscium* (Miwatani and Shinoda, 1971; Shinoda *et al.*, 1976).

Concurrently, other investigators reported that anti-H serum raised in rabbits immunized with intact polar flagella of *V. parahaemolyticus* failed to agglutinate *V. cholerae*, *V. anguillarum* tested by H-agglutination (Sakazaki *et al.*, 1970; Smith, 1974). As a consequence, Shinoda *et al.* (1980) reexamined these seemingly opposite findings and demonstrated that, in fact, there were two types of antigenic determinants of the polar flagellum. One was on the surface, so called surface antigenic determinant (SA). The other was buried inside the flagellum and would be exposed when the flagellum was dissociated to flagellin monomers. The latter was named internal antigenic determinant (IA). Therefore, SA on the intact flagellum of *V. parahaemolyticus* was different from the other strains but shared common antigens of IA on the flagellin monomers with *V. cholerae* and *V. anguillarum*. In conclusion, SA was responsible for H-agglutination reaction whereas IA was responsible for the reaction in gel diffusion test. In addition, they also demonstrated that the *V. parahaemolyticus* monomers did not possess SA because they did not inhibit H-agglutination.

In this investigation, our objectives was to produce and characterize monoclonal antibodies (MAb) against polar flagella of *V. parahaemolyticus*. Subsequently, these MAbs will be used as tools in adhesion studies of *V. parahaemolyticus* to the rabbit intestinal mucosa and in the isolation of the corresponding gene(s) of the bacterial flagella.

MATERIALS AND METHODS

Bacterial strains and media : The bacterial strain used in this study was the motile *Vibrio parahaemolyticus* ATCC 10136. Maintenance of stock culture was carried out monthly in a maintenance medium (8 g Tryptone, 20 g NaCl, 4 g nutrient broth, 4 g $MgCl_2$, 4 g KCl, and 4 g agar) throughout this study. For purification, the bacterium was grown in alkaline peptone broth (10 g Bacto-peptone, 20 g NaCl, 2 g Yeast extract, 2 g KCl, and 2 g $MgCl_2.6H_2O$, pH 7.2-7.6) at 37°C for 16-18 hrs with vigorous shaking at 200 rpm.

Purification of polar flagellar core protein : Isolation and purification of polar flagella was modified from the method described by Yang *et al.* (1977) for purification of flagellar core proteins of *V. cholerae*. Briefly, two liters of an overnight culture of *V. parahaemolyticus* (4×10^8 cells/ml) were sedimented and the cell pellets were resuspended in 0.15 NaCl. The cell suspension was chilled on ice for 15 to 30 min and the polar flagella were sheared in a Waring blendor. Several rounds of differential centrifugation were applied to the supernatant, obtained from the previous step, to ensure that no flagellar sheath was observed. Finally, the partially purified flagella preparation was applied to a cesium chloride (CsCl) isopycnic centrifugation. The flagellar band was removed from the gradients and dialysed against TE (10 mM Tris, 5 mM EDTA, pH 8.0) buffer at 4°C with several changes. After dialysis, the band was examined under a transmission electron microscope

(TEM) (JEOL 100CX) and the sample was stored at 4°C. Bicinchoninic Acid protein determination (BCA) (Pierce, Rockford, IL) was done to determine the concentration of the purified flagellar core protein.

Immunization protocol : Five BALB/c mice were immunized intraperitoneally with 100 g of purified flagella at 2-week intervals. One month following the first immunization, the mice were boosted with 100 g of flagellar protein and their spleens were used in the fusion experiment 3-4 days later.

Hybridoma production : The protocol for fusion of spleen cells with myeloma cells was based on the method reported by Oi and Herzenberg (1980) and modififed by Adams *et al.* (1988). Briefly, mouse spleen cells were fused with myeloma line Sp2/0 in the presence of polyethylene glycol 1500 (Boehringer Mennheim, West Germany). Following fusion, the resulting hybridomas were screened for production of antibodies reactive with purified flagellar core by the enzyme-linked immunosorbent assay (ELISA). Each hybridoma secreting antibody was cloned by three limiting dilutions on a mouse thymocyte feeder layer and they were retested for anti-PH reactivity. Cloned hybridomas were expanded in tissue culture and 10^7 hybridoma cells were injected into pristane-primed mice for ascites production (Simonson and Siebeling, 1988).

Characterization of monoclonal antibodies (MAb) : The MAb immunoglobulin (Ig) in the ascites and cell culture supernatant fluid were isotyped by Ouchterlony test (The Binding Site Inc., San Diego, CA) and clarified by centrifugation. Subsequently, the Ig fractions were precipitated by $(NH_4)_2SO_4$. Following dialysis, IgM MAbs were purified by anti-mouse IgM (-chain specific)-agarose column chromatography (Sigma Chemical Co., St. Louis, Mo.). The IgG MAbs isotypes were affinity purified on Protein A-Sepharose (Sigma Chemical Co., St. Louis, Mo.). The purified MAb Igs were concentrated by the Amicon filtration system (Amicon Inc., Bevery, MA) or by Aquacide dehydration (Calbiochem, La Jolla, CA) and protein concentrations were determined by the BCA assay.

Preparation of coagglutination reagents : IgG MAbs were adfixed through the Fc region to formalin-killed *Staphylococcus aureus* Cowan I ATCC 12598 cells. To determine the quantity of MAb required, equal volumes of two-fold dilutions of purified IgG MAbs starting with at least 0.5 g/ml of antibody and 10% suspension (vol/vol) of prepared *Staphylococcus* cells (Simonson and Siebeling, 1986) were mixed, incubated at 4°C overnight, and the IgG-armed *S. aureus* were sedimented. The armed cells were suspended in 2.5 volumes of 0.067 M phosphate buffer saline, pH 7.2 (PBS). The titer was determined by slide agglutination and appropriate dilutions were chosen to prepare a large-scaled coagglutination reagents.

For IgM MAbs, 0.2-m unmodified latex beads (Sigma Chemical Co., St. Louis, Mo.) were used as carriers. The latex beads were washed and standardized in glycine bufferred saline (GBS; 7.3 g glycine, 10 g NaCl, and 1 g sodium azide, pH 8.2), and a working dilution was determined as above. Similarly, equal volumes of two-fold diluted antibody and standardized latex beads were mixed, incubated in 37°C-water bath for 2 hrs. The IgM-coated latex beads were sedimented and resuspended in 1 volume of GBS-bovine serum albumin (GBS-BSA; 0.1 g BSA in 100 ml GBS) and stored at 4°C until needed. The IgM concentration that gave an optimum agglutination reactions was chosen to produce a large batch of the latex reagent.

Coagglutination tests : Thirty-five strains of *Vibrio parahaemolyticus* and thirty-four heterologous *Vibrio* species were tested. Each *Vibrio* isolate was grown on an alkaline peptone

agar slant at 37°C for 16-18 hrs. The cells were harvested in 1-2 ml of TET buffer for 1 and 24 hrs and slide agglutination tests were carried out.

Immunoelectron microscopy : The method employed was modified from that described by Adams *et al.* (1988). Cell suspension in PBS (10^5 - 10^6 cells/ml) was used as antigen and the grid was blocked with 2% bovine serum albumin (BSA) and incubated with the supernatant from antibody-secreting hybridoma or affinity-purified Ig. Then a gold labelled goat anti-mouse immunoglobulin IgG+IgM (H+L) (10-nm colloidal gold particles) (Amersham, UK) diluted 1:15 in PBS was applied to the grid. Finally, the grid was washed with PBS and distilled water and examined under TEM. In case of negative staining, 1% uranyl acetate was applied after removal of cell suspension fluid.

When polar flagellar core was stained, the flagellar sheath was removed to expose the core protein. To do so, *V. parahaemolyticus* ATCC 10136 was grown in 4-ml alkaline peptone broth at 37°C for 16-18 hrs. The cells were then sedimented and resuspended in 3-4 ml of saline solution. The cell suspension was placed on a carbon-coated grid and treated in the following manner; N/100 HCl at 5, 10, 15, and 30 min; 6 M Urea at 5, 10, 15, and 30 min; or autolysis at 4°C. At each time interval, a portion was removed and examined under the TEM (Follett and Gordon, 1963). Only those preparations shown no sheath were used in immunoelectron microscopy as described above.

RESULTS

Purification of polar flagellar core protein : The polar flagellar core (flc) antigen of *V. parahaemolyticus* was purified from overnight culture in alkaline peptone broth. The flagella were sheared and followed by differential centrifugation. They were further purified by CsCl gradients centrifugation in TET buffer. Triton X-100 has been a successful treatment to remove the flagellar sheath of *V. cholerae* (Ferris *et al.*, 1984) and those of *Bdellovibrio bacteriovorus* (Thomashow and Rittenberg, 1985). Furthermore it also dissociated the contaminating vesicles present in the crude preparation as well as in the CsCl gradients step (Yang *et al.*, 1977). Four to five cycles of differential centrifugation were required to effectively remove the flagellar sheath. In the absence of Triton X-100, the vesicles became bound to the flagellar core and contaminated this organelle in the CsCl gradient bands. A transmission electron micrograph of purified polar flagella revealed that they were free of cell debris and vesicles (Figure 1).

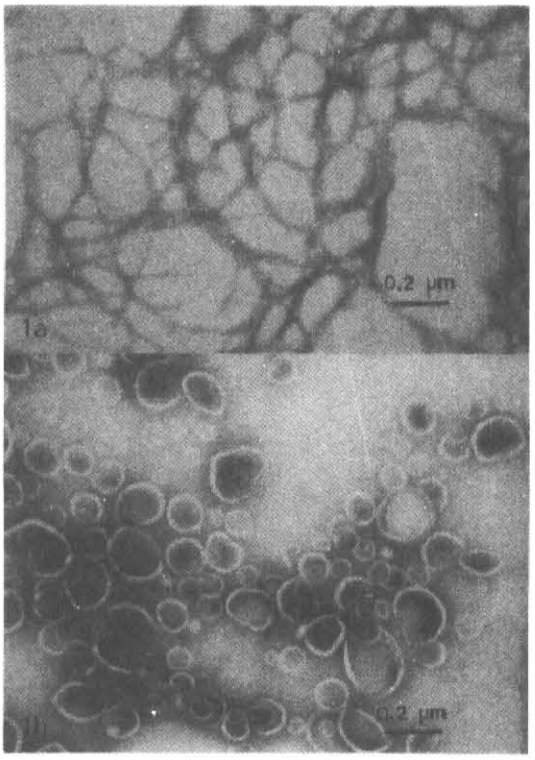

Figure 1 Transmission electron micrograph of purified polar flagellar core protein by CsCl gradients centrifugation (1a) devoid of unidentified vesicles (1b).

Production and characterization of the anti-polar flagellar monoclonal antibodies: From two fusion experiments, twelve hybridomas which secreted MAbs reacting with *V. parahaemolyticus* core protein were identified by ELISA (OD_{405} > 1.0). One clone designated 4G7 was selected, cloned, and maintained as a source of anti-core MAb (MAb-flc) for subsequent experiments while the remaining clones were frozen and stored as back-up clones. Hybridoma clone 4G7 secreted IgG1 MAb which reacted specifically with the polar flagellar core by immunogold labelling (Figure 2).

The removal of the sheath, which enshrouds the core, was attempted by subjecting whole flagella to each of the following treatments 1) N/100 HCl at pH 2, 2) 6 M Urea, or 3) autolysis in saline at 4°C. Each of the three treatments did not appear to affect the structural integrity of the core which is similar to that reported for *V. cholerae* El Tor and *V. metschnikovii* (Follett and Gordon, 1963). However, the degree of sheath dissociation varied among the different treatments.

Neither HCl treatment nor exposure to 6 M urea successfully removed the sheath. The HCl treatment for 5 and 10 min showed the same evidence of flagellar sheath disintegration. Exposure the flagella to HCl for 15 min removed the entire sheath (however most flagella became detached from the cell bodies) and at 30 min exposure prevented adherence of the cells to the grids. When vibrio cells were treated with urea they failed to adhere to the grid at any treatment time period, which may reflect the instability of the cells in urea solution. Interestingly, when a saline *V. parahaemolyticus* cell suspension was incubated at 4°C for 9 days, the flagellar sheath was removed completely. Figure 3a shows cells incubated for 2 days at 4°C with no evidence of sheath loss. However, following five days incubation, the sheath appears to open up longitudinally. In some microscopic fields, the intact core were partially visualized (Figure 3b), and after nine days of incubation at 4°C, the entire flagellar core could be seen (Figure 3c). During the 4°C-incubation, individual cells autolyzed, but the cellular debris remained attached to the flagella. In one preparation of purified flagellar core, the sheath was not completely removed because there were not enough cycles of differential centrifugation. Therefore the fusion using this preparation to immunize mice and to screen hybridomas resulted in anti-sheath (MAb-

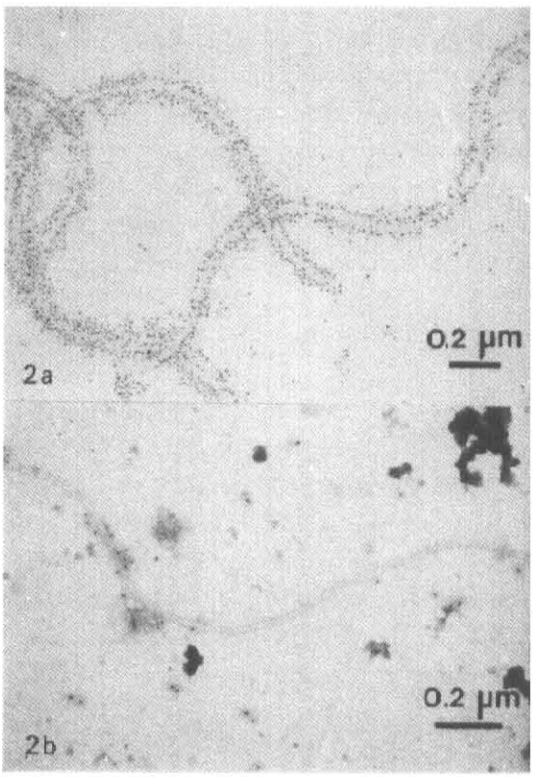

Figure 2 Transmission electron micrograph of positive reaction of monoclonal antibody against *V. parahaemolyticus* polar flagellar core protein by immunogold labelling (2a) compared to normal mouse serum as a negative control (2b).

fls) instead of anti-core. However, a decision to use these MAbs was made.

Two hybridomas, 3C12 and 1A9, which secreted anti-polar flagellar sheath MAb (MAb-fls) were generated, both of which secreted IgG2a isotype. Ascitic fluid was produced for the following experiments. The immunogold labelling was carried out in similar manner except that the bacterial cells were suspended in saline solution and incubated at 4°C for only 1-2 days. Figure 4 illustrates the reaction of the MAb to only the sheath but not to the exposed intact core protein.

Coagglutination reactions were done by slide agglutination with 35 *Vibrio parahaemolyticus* strains (Table 1) as well as 34 heterologous *Vibrio* species (Table 2). Coagglutination reagent for Mab-flc reacted with every strain of *V. parahaemolytius* as expected since the polar flagellar antigens have been reported to be species-specific for the genus *Vibrio* (Bhattacharyya, 1975; Bhattacharyya and Mukerjee, 1974; Simonson and Siebeling, 1986; Tassin *et al.*, 1984).

Several strains of *V. parahaemolyticus* suspended in PBS were compared to those suspended in TET. Cells suspended in TET reacted

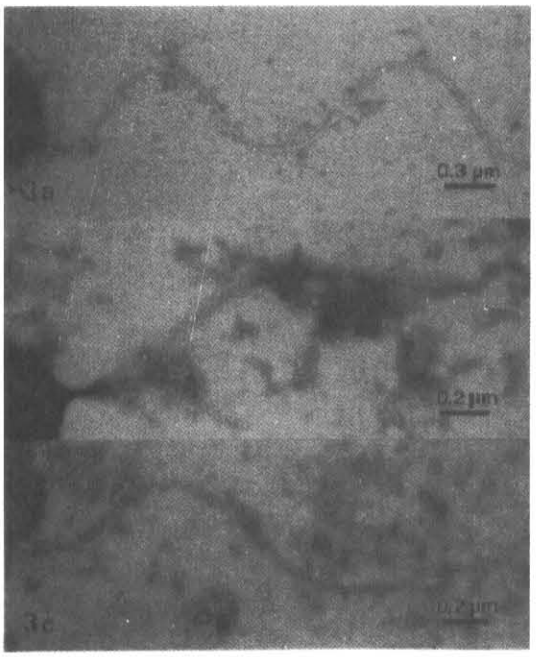

Figure 3 *V. parahaemolyticus* cell suspension in saline solution at 4C. Two days after incubation, the sheath was still intact (3a). Five days after incubation, the sheath appeared to partially open longitudinally (3b) and was completely removed at day nine (3c).

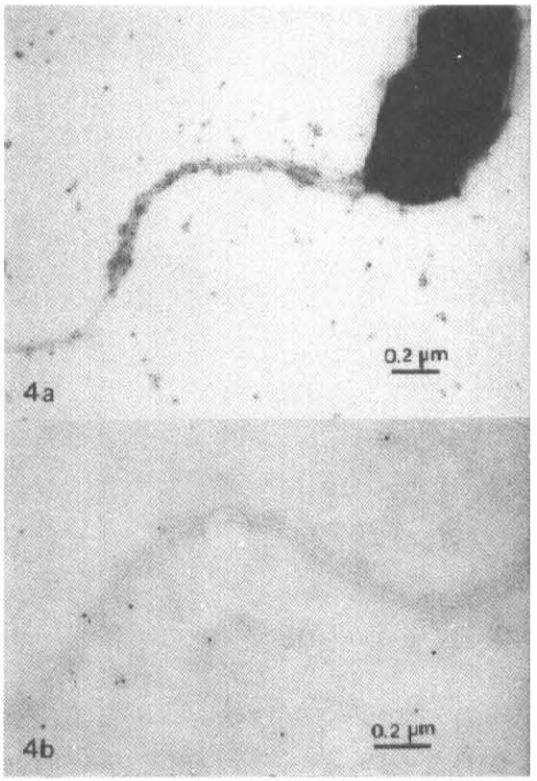

Figure 4 Transmission electron micrograph of the positive reaction of monoclonal antibody against polar flagellar sheath by immunogold labelling (4a) while the MAb did not react with the core protein (4b).

Table 1 Coagglutination reactions for thirty five *Vibrio parahaemolyticus* strains tested with *Staphylococcus* cells armed with flagellar-core-specific monoclonal antibody (MAb-flc) and with sheath-specific monoclonal antibody (MAb-fls).

V. parahaemolyticus strains	MAb-Fc	MAb-Fs
L5	+ +	+ +
L7	+ + + +	+ + + +
L8	+ + + +	+ + + +
L9	+ + + +	+ +
L20	+ + + +	+ + +
L21	+ + + +	+ + +
L22	+ + + +	+ + +
L31	+ + + +	+ + + +
L36	+ + + +	+ + + +
L37	+ + + +	+ + + +
L41	+ + +	+
L84	+ +	+
L90	+ + + +	+ + + +
L97	+ + + +	+ + +
L101	+ + + +	+ + + +
L102	+ + ++	+ + + +
L104	+ + + +	+ +
L109	+ + + +	+ + +
L118	+ + + +	+ + + +
L126	+ + + +	+ + + +
L135	+ + + +	+ + + +
L140	+ + + +	+ + +
L145	+ + + +	+ + +
L1450	+ + + +	+ + + +
L145T	+ + +	+ + +
L30Y	+ + +	+ +
L86Y	+ + + +	+ + + +
L87Y	+ + + +	+ + + +
L95Y	+ + +	+ + +
L96Y	+ + + +	+ + + +
L117Y	+ + + +	+ + +
L122Y	+ + + +	+ +
L144Y	+ + + +	+ + +
L148Y	+ + + +	+ + + +
ATCC 10136	+ + +	+ + + +

Agglutination reactions were examined and scored to indicate the agglutination within 30 sec (+ + + +), 30 sec to 1 min (+ + +), 1 to 2 min (+ +), 2 to 3 min (+), or no agglutination (-).

Table 2 Coagglutination reactions for thirty four heterologous *Vibrio* species tested with *Staphylococcus* cells armed with flagellar-core-specific monoclonal antibody(MAb-flc) and with sheath-specific monoclonal antibody (MAb-fls).

Vibrio species	MAb-flc	MAb-fls
V. aestaurianus ATCC 35048	0	0
V. albensis	0	0
V. alginolyticus ATCC 33787	+ + + + +	
V. anguillarum ATCC 19264	0 0	
V. anguillarum DOI	0	0
V. campbellii ATCC 25920	0	+ + + +
V. carchariae ATCC 35084	0	+ + +
V. cholerae LA 5875	0	0
V. cholerae ATCC 14035	0	0
V. cincinnatiensis ATCC 35912	0	0
V. costicola NCMB 701	0	0
V. damsela ATCC 35016	0	0
V. diazotrophicus ATCC 33466	0	0
V. fischeri NCMB 1281	0	0
V. fluvialis ATCC 33810	0	0
V. furnissii ATCC 35016	0	0
V. gazogenes ATCC 29988	0	0
V. harveyi NCMB 1280	0	+ + +
V. hollisae ATCC 33564	0	0
V. mediterranei ATCC 43341	0	0
V. metschnikovii ATCC 7708	0	0
V. mimicus ATCC 33653	0	0
V. natriegens ATCC 14048	0	+ + + +
V. neresis ATCC 25917	0	0
V. ordalii ATCC 33509	0	0
V. orientalis ATCC 33934	0	0
V. pelagius I ATCC 25916	0	0
V. proteolyticus NCMB 1326	0	0
V. splendidus I ATCC 33125	0	0
V. splendidus II ATCC 25914	0	0
V. tubiashii ATCC 19105	0	0
V. tubiashii ATCC 19106	0	0
V. vulnificus I ATCC 27562	0	+ + +
V. vulnificus II ATCC 33147	0	+ +

Agglutination reactions were examined and scored to indicate the agglutination within 30 sec (+ + + +), 30 sec to 1 min (+ + +), 1 to 2 min (+ +), 2 to 3 min (+), or no agglutination (-).

faster with MAb-flc than those suspended in PBS. This was not unexpected since the core antigen is located beneath the sheath. Therefore, in order for MAb-flc to react with the core, the antibody had to penetrate the sheath barrier. Treatment with Triton X-100 removed the sheath and provided access to the antigen. Most of *V. parahaemolyticus* strains, which are environmental isolates strongly reacted with MAb-flc within the first minute. A few strains (L5, L84) agglutinated in the second minute.

MAb-fls raised against flagellar sheath exhibited similar strong reaction with its homologous species. As shown in Table 2, most of them agglutinated within the first minute, for example, L7, L8, L36, L101, and L86Y. Interestingly, the strains that exhibited weak reactions with MAb-flc, also reacted in relatively the same manner as MAb-fls such as L5, L84. Seven species other than the vaccine species reacted with the MAb-fls including *V. alginolyticus, V. campbellii, V. carchariae, V. cincinnatiensis, V. harveyi, V. natriegens, V. vulnificus* I *and V. vulnificus* II. The sheath antigen, yet to be defined, was not species-specific as the core protein. Furthermore, when anti-core reagents were tested, the vibrio cell suspension was incubated in TET buffer to facilitate removal of sheath and to expose flagellar epitopes while anti-sheath reagents agglutinated vibrio cell suspensions in either PBS or TET buffer.

DISCUSSION

In this investigation, a rapid simplified method to purify flagellar core protein from *V. parahaemolyticus* ATCC 10136 was developed. The flagellar core preparation showed long flagellar filament similar in morphology to those described by Miwatani *et al.* (1970) and Shinoda *et al.* (1970). Prior to CsCl gradient centrifugation, vesicles contaminated the crude flagellar preparation and they could be eliminated by treatment with Triton X-100. In the absence of this detergent, the spherical bodies still contained in the gradient. In the method reported here, two bands were observed following CsCl centrifugation, an upper band which contained purified flagella and the lower band that contained vesicles. The lower band could be removed without contaminating the flagellar band.

Several fusion experiments were prepared to obtain MAbs with serological activity against flagellar core protein. Previously, anti-flagellar serum was raised against either intact flagella (Tassin *et al.*, 1983) or flagellin monomers (Shinoda *et al.*, 1970; Shinoda *et al.*, 1976) in rabbits. Polyclonal anti-H antibody showed species specificity (Sakazaki *et al.*, 1970) when tested against formalin-fixed whole cell preparations and purified flagellin protein (Shinoda *et al.*, 1976). In this investigation, purified intact flagella was used as the antigen for MAb production. After testing MAb-flc with 35 strains of *V. parahaemolyticus*, the MAb-flc exhibited species specificity. Therefore this result agreed with the previous finding which indicated this antigen the surface antigen (SA) from intact flagella.

MAbs specific for polar flagellar sheath were also obtained in this study. The flagellar sheath epitopes expressed by *V. parahaemolyticus* were also detected on *V. alginolyticus, V. campbellii, V. carchariae, V. cincinnatiensis, V. harveyi, V. natriegens* and *V. vulnificus*. It was not surprising that both MAb-flc and MAb-fls reacted with *V. alginolyticus* since this organism is biochemically similar to *V. parahaemolyticus*. *V. alginolyticus* was originally described as a biotype of *V. parahaemolyticus*. Both species produce a sheathed single polar and numerous unsheathed lateral flagella (Bauman *et al.*, 1984). The flagellin protein of *V. alginolyticus* from polar as well as lateral flagella were reported to be serologically similar to those from polar and lateral flagella of *V.*

parahaemolyticus, respectively (Miwatani and Shinoda, 1971; Shinoda *et al.*, 1976).

The polar flagellum of *V. parahaemolyticus* is 24-30 nm in diameter and consists of a core (14 to 16 nm) surrounded by a sheath (Bauman *et al.*, 1984) which has been reported to be continuous with the outer membrane of the cell wall not only for *V. parahaemolyticus* but also in other *Vibrio* species (Allen and Bauman, 1971; Follet and Gordon, 1963) and *Bdellovibrio bacteriovorus* as well. It was reported that for *V. cholerae*, the sheath and outer membrane contain a common protein composed of three polypeptides with molecular weights of 60 kD which are not related serologically to the flagellin protein. Antibody produced against the lipopolysaccharide (LPS) of *V. cholerae* reacted with the outer membrane only, not the sheath (Bauman *et al.*, 1984). However, this finding was contrary to that of Adams *et al.* (1988) when monoclonal antibodies against *V. cholerae* O1 lipopolysaccharide were produced. They reported that these monoclonal antibodies reacted with both cell surface and the flagellar sheath as demonstrated by immunoelectron microscopy. Studies done on the composition of the flagellar sheath from *Bdellovibrio bacteriovorus* showed that it contained approximately 25% protein, 38% phospholipid, and 12% LPS (Thomashow and Rittenberg, 1985). They also reported that the flagellar sheath from this organism was a stable domain, distinct from the bulk of the outer membrane constituents. However in this research, MAb-fls remains to be determined. Additional investigations may identify whether the epitope that MAb-fls recognizes is protein or lipopolysaccharide.

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